Evolution of Queensland Spiny Mountain Crayfish of the Genus *Euastacus* Clark (Decapoda: Parastacidae): Preliminary 16s mtDNA Phylogeny

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The upland, mesic rainforests of Queensland are scattered in a series of disjunct montane blocks along the east coast. They are remnants of a once widespread forest type that dominated Australia during the Miocene. Each of these montane blocks harbours a unique species of spiny mountain crayfish belonging to the genus *Euastacus* (Parastacidae: Decapoda).

The preliminary 16s mtDNA data shows some incongruence between the phylogenetic relatedness and the geographic location of some species. To account for this a multiple range expansion-vicariance hypothesis is proposed. It is postulated that the group evolved from the range expansion of a southern ancestral form. This form underwent a range contraction that resulted in at least two ancestral Queensland forms. These underwent a subsequent range expansion. A shift in climate, probably associated with the late Miocene drying of the continent, resulted in the ranges of these ancestral forms receding to upland areas. This isolation resulted in a large number of different species each with restricted distributions.

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Key words: *Euastacus*, freshwater crayfish, evolution, mtDNA, phylogeny.

INTRODUCTION

The upland rainforests of Queensland are scattered along the east coast in disjunct blocks. There is a relatively large, fragmented block on the mountain ranges in the south-east, a few small remnants on the isolated mountains of the central coast, and another relatively large, fragmented block on the mountain ranges in the Wet Tropics. The distribution of these upland rainforest areas has been described as a 'mesothermal archipelago' as they constitute a chain of temperate mountain and tableland 'islands' that rise from a 'sea' of tropical and subtropical lowlands (Nix 1991). These mesic areas are remnants of a once more widespread forest type that dominated Australia during the early Miocene (Moritz et al. 1997 and references therein). It has been suggested that the historical expansion, contraction and fragmentation of these refugia played an important role in the speciation of rainforest restricted taxa (Heatwole 1987).

Most of the mesic rainforest refugia of Queensland harbour a unique species of spiny mountain freshwater crayfish of the genus *Euastacus* (Morgan 1988, 1989; Horwitz 1990; Short and Davie 1993). There are now fifteen species described from Queensland (Short and Davie 1993). All but one, *E. suttoni*, which inhabits streams of the granite belt on the New England Tableland, inhabit the cool, fast flowing, well shaded streams of montane rainforests (Morgan 1988, 1989).

This cold water adapted group (Riek 1969) is confined to higher altitudes as latitudes decrease. In south-east Queensland they occur only above 250 m, in central Queensland

above 750 m, and in north Queensland above 900 m (Morgan 1988, 1989; Cannon and Sewell 1994: 35, fig. 1). Riek (1959), noticing this interesting distribution, postulated that the group probably had a wide-ranging common ancestor that, once climatic conditions became hotter and drier, receded to the cooler mountains. This isolation resulted in a large number of different species each with restricted distributions.

Morgan (1986, 1988, 1989, 1997), in the latest revision of the group, agreed that allopatric speciation was probably the dominant process of speciation. However, he stopped short of a detailed phylogeny or a detailed discussion of speciation. He identified only one morphological character, the presence or absence of a male cuticle partition, that may be useful in inferring phylogenetic relationships, although he was uncertain if this character was reliable.

To understand the evolutionary diversification of this group it is imperative to have a robust phylogeny. The unique features of mtDNA which predispose it to evolutionary studies have been well documented (see Moritz et al. 1987; Avise 1994).

This paper presents a preliminary 16s mtDNA phylogeny for eight of the fifteen species of *Euastacus* found in Queensland. We propose a hypothesis that may explain their evolution. Finally, we detail our current research which we hope, combined with a full phylogeny of the genus, will shed light on the evolution of these crayfish.

MATERIALS AND METHODS

Collection of Samples

Samples of *E. robertsi*, *E. fleckeri*, *E. balanensis*, *E. eungella*, *E. hystricosus*, *E. setosus*, and *E. sulcatus* were collected from the field with a dip net (see Table 1), transferred to liquid nitrogen for transportation, and once in the lab stored at –75°C. Specimens were identified using Morgan's (1988, 1989) keys. Voucher specimens will be lodged with the Queensland Museum once this study is completed.

Samples of *E. suttoni* were provided by Adrian Dawson. Tissue samples of *E. spinifer* were obtained from alcohol preserved samples from the Queensland Museum. Total genomic DNA of *Cherax quadricarinatus* was provided by Peter Mather, Queensland University of Technology.

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted by placing approximately 50 mg of muscle tissue in 350 l of extraction buffer (0.5 M Tris pH 8.0; 0.25 M NaCl: 0.025 M EDTA: 0.175 mM SDS) and 1 μl Proteinase K and incubated overnight at 55°C. Samples that were stored in alcohol were first soaked in STE for 10 mins. Proteins and lipids were removed by sequential extractions with equal volumes of phenol, phenol-chloroform-isoamyl alcohol (25: 24: 1) and chloroform-isoamyl alcohol (24: 1). DNA was precipitated at 4°C in 3 M sodium acetate (1/3 volume) and isoproponol (1 volume). Pellets were washed in 70% ethanol, dried and resuspended in 50 μl of TE buffer (10 Tris HCl, 1 mM EDTA, pH 7.5).

The 16s ar and br primer set (Simon et al. 1994) was used to amplify a 461 base pair section of the 16s mtDNA fragment. PCR reactions contained 30 nMol each of dATP, dGTP, dCTP and dTTP (Promega), one unit of TAQ DNA polymerase (Promega), 2.5 mMol $MgCl_2$ (Promega), 5 μl of 10 x polymerase reaction buffer (Promega), 0.5 μMol of each primer, 0.2 μg of template DNA and adjusted to a final volume of 50 l. DNA was initially denatured at 95°C for 5 min, then 35 cycles of 94°C denaturing for 1 min, 50°C annealing for 30 sec, and 72°C extension for 1 min, followed by a final 68°C extension step for 5 min.

TABLE 1

Collecting location of samples.

Specimen	Collecting Locality
E. robertsi	Hilda Ck., Thornton Peak
E. fleckeri	Paul's Luck, Mt Spurgeon
E. balanensis	Kauri Ck., Lamb Range
E. eungella	Cattle Ck., Clarke Range
E. hystricosus	Bundaroo Ck., Conandale Range
E. setosus	Greens Falls, Mt Glorious
E. sulcatus	Canungra Ck., Lamington Plateau

Three μl of the resulting PCR product was run on agarose gels and visualised using ethidium bromide. The remaining product was cleaned using Bresaclean (Bresatek), following the manufacturer's instructions. Sequencing reactions were carried out using dye terminator cycle sequencing reactions (Perkin Elmer) as per manufacturer's instructions. 30–40 ng of cleaned template DNA and 3.2 pmol of primer were added. Sequencing was carried out on an Applied Biosystems 373 automated sequencing machine. Both the light and heavy strands were sequenced.

Sequence Alignment and Phylogenetic Analysis

Sequences for *E. bispinosus*, *Cherax destructor albidus* (formerly *C. albidus*, see Campbell et al. 1994) and *Geocharax gracilis*, published by Crandal et al. (1995), were incorporated into the analysis. Sequences were aligned with the program Clustal V (Higgins 1992).

Genetic distances between sequences were calculated with Mega (Kumar et al. 1993) using the Tamura-Nei distance estimation model. Maximum likelihood analysis was performed using DNAML in Phylip (Felsenstein 1993) incorporating a transitions to transversions ratio of two. Unweighted maximum parsimony analysis was performed using the branch and bound search option in PAUP (Swofford 1993). Neighbour joining analysis was performed using Mega (Kumar et al. 1993) with a Tamura-Nei distance estimation model. Neighbour joining trees were constructed with gaps and missing information treated as both complete deletions and as pairwise deletions. Bootstrap values were calculated using 1,000 replications. *G. gracilis*, *C. quadricarinatus* and *C. d. albidus* were used as outgroups.

RESULTS

The genetic differentiation between species is presented in Table 2. The phylogram generated by the maximum likelihood method, and geographic location of each species, is presented in Fig. 1. It appears that the Queensland *Euastacus* examined are monophyletic (bootstrap 79%) to the exclusion of the southern species *E. spinifer* and *E. bispinosus*. Within this group there are three well supported clades: *E. robertsi/E.balanensis* (bootstrap 100%), *E. eungella/E. setosus* (bootstrap 70%) and *E. hystricosus/E. sulcatus* (bootstrap 74%). All three methods of phylogenetic analysis generated these clades. There is incongruence between the phylogenetic relatedness and geographic location of *E. eungella* and *E. setosus*. These two species clade together but occupy ranges which are geographically distant.

TABLE 2

Sequence divergences generated using Tamura-Nei distance estimation model.

	2	3	4	5	6	7	8	9	10	11	12	13
1	.0277	.0813	.0966	.0930	.1028	.0983	.1172	.1161	.1741	.2460	.2488	.2919
2		.0737	.0946	.0967	.0891	.1021	.1091	.1051	.1622	.2530	.2323	.2655
3			.0421	.0675	.0514	.0597	.0676	.0730	.1389	.2671	.2101	.2507
4				.0643	.0414	.0698	.0672	.0749	.1268	.2756	.2382	.2466
5					.0803	.0471	.0652	.0835	.1449	.2462	.2366	.2627
6						.0746	.0651	.0596	.1405	.2632	.2377	.2560
7							.0577	.0676	.1417	.2430	.2269	.2633
8								.0603	.1492	.2377	.2333	.2613
9									.1007	.2285	.2215	.2530
10										.2673	.1762	.2187
11											.2671	.3139
12												.2425

1. *E. robertsi*, 2. *E. fleckeri*, 3. *E. balanensis*, 4. *E. eungella*, 5. *E. hystricosus*, 6. *E. setosus*, 7. *E. sulcatus*, 8. *E. suttoni*, 9. *E. spinifer*, 10. *E. bispinosus*, 11. *C. quadricarinatus*, 12. *E. C. d. albidus*, 13. *G. gracilis*.

DISCUSSION

There are at least three hypotheses that can be erected to explain the evolution of the Queensland *Euastacus*; and because the mode and tempo of evolution will determine the shape of the phylogeny (Purvis 1996 and references therein) there are at least three topologically different hypothetical phylogenies.

The first is a vicariance hypothesis. Once the ancestral *Euastacus* evolved it spread rapidly along the east coast of Australia during periods when favourable habitat prevailed (wet and cool) and when there were few barriers to dispersal. Once climate changed (warm and/or dry), the range of the ancestral *Euastacus* receded up the mountain ranges. Vicariance resulted in allopatric speciation. If this hypothesis is valid, one would expect neighbouring species to be more phylogenetically similar to each other than they are to geographically distant ones. Furthermore, if a shift in climate resulted in simultaneous vicariance events across the whole of the ancestral range, one would expect similar branch lengths leading to the tips of clades. There should be very few internodes, and for the internodes that are present, the branch lengths separating internodes should be very short. The tree should also be balanced.

The second hypothesis is stepwise colonisation. Once the ancestral *Euastacus* evolved, there was a progressive stepwise colonisation and isolation. In this scenario, too, one would expect that there would be geographic and phylogenetic congruity. In this scenario, though, one would expect a well delineated basal clade, giving rise to another clade, which in turn is basal to another clade. The topology of the tree would not be symmetrical because the more derived clades would have much shorter branch lengths. The basal clades should correspond to where the ancestral *Euastacus* evolved. If the ancestral *Euastacus* evolved in southern Australia, one would expect the southern species to form the basal clades of the phylogeny and the northern species to be derived from them.

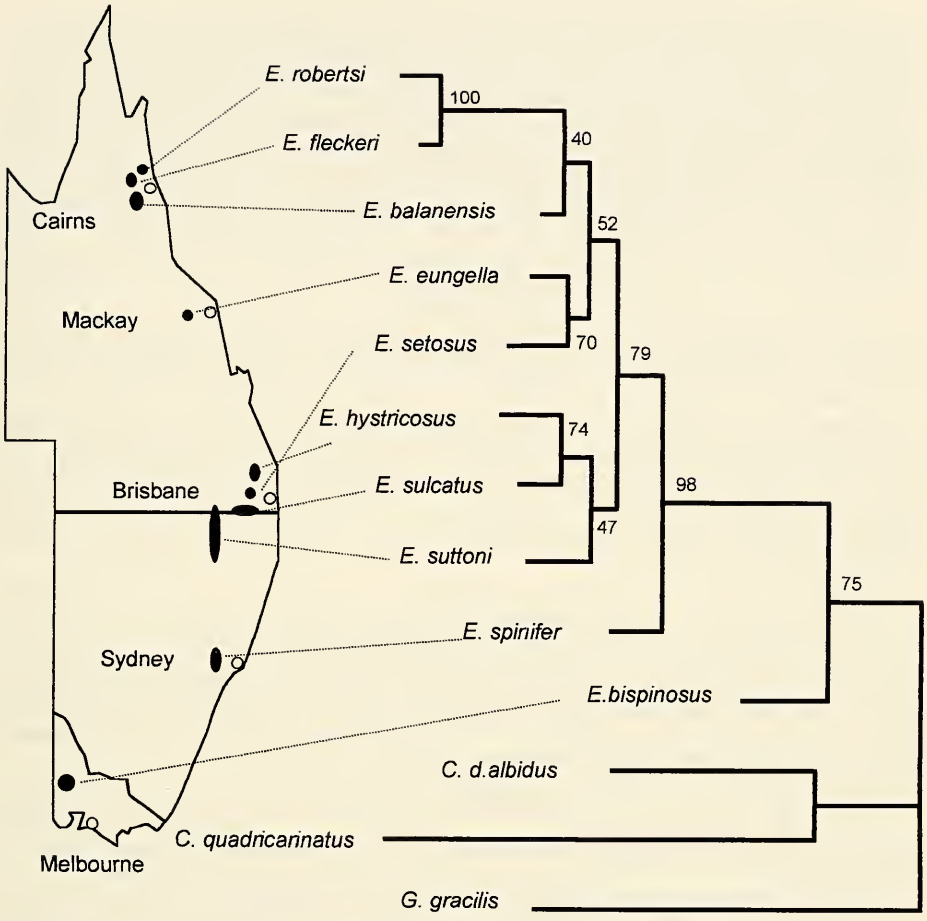

Figure 1. 16s mtDNA maximum likelihood phylogeny showing geographic location of each species on a schematic map. Neighbour joining bootstrap values have been included.

In the third hypothesis the evolution of the group may be accounted for by multiple range expansions and vicariance events. The ancestral *Euastacus* once evolved, extends its range, then contracts leading to vicariance. These new forms extend their range when conditions are suitable, and then contract again when conditions are not favourable. In this scenario, one would not expect absolute congruence between geography and phylogenetic relatedness. It is expected there would be well defined lineages that correspond to the periods of isolation. There should be some structure evident where closely related species would be geographical neighbours. Importantly, not all geographically neighbouring species would be closely related to each other.

The most plausible of the three hypotheses seems to be the multiple range expansion-vicariance hypothesis because there is some incongruence between the phylogenetic relatedness and geographic location of some species. These hypotheses can not be tested because the 16s phylogram is generated from a preliminary data set and is not fully resolved.

There are, though, a few further points worth considering. First, *E. bispinosus* (a Victorian species) is the basal clade of the phylogram generated. It has long been postulated that southern Australia was the centre of origin of the *Euastacus* (Riek 1969).

Second, the genetic distances between species indicate differentiation in the late Miocene or early Pliocene. Moritz et al. (1997) have outlined some of their work on the speciation of a number of endemic Queensland rainforest-restricted vertebrate taxa. Using the same fragment of 16s mtDNA, and assuming a 16s molecular clock that had genetic divergences of 1% roughly equating to 1 million years (C. Moritz pers. comm.), they tentatively postulated that speciation within these groups probably occurred during the Miocene or early Pliocene. If we apply the same clock to this data set (noting that the clock has not been calibrated nor developed for invertebrates), we get similar figures indicating differentiation in the late Miocene or early Pliocene. These speciation events may coincide with the late Miocene contraction of mesic rainforests (Moritz et al. 1997). Interestingly, there is a *Euastacus*-like fossil Parastacid from the Eocene (Sokol 1987).

Based on the preliminary 16s mtDNA data, a hypothesis can be erected to explain the possible evolution of the Queensland *Euastacus*. There was a range expansion of a southern *Euastacus* into Queensland. A subsequent range contraction resulted in at least two ancestral Queensland forms. These underwent a subsequent range expansion. A shift in climate, possibly the late Miocene drying, resulted in allopatric speciation of these ancestral forms.

The main reason for proposing a multiple range expansion-vicariance hypothesis for this group is that *E. setosus*, whilst being geographically closest to *E. hystricosus* and *E. sulcatus*, is phylogenetically closest to *E. eungella*. Another interpretation which may account for this incongruence is that the phylogram does not accurately reflect the evolutionary diversification of this group. Other genetic studies on crayfish (see Crandall and Fitzpatrick 1996; Crandall et al. 1995; and Avery and Austin 1996) found inconsistencies between the genetic and morphological data. In this case, however, the genetic data are consistent with Morgan's (1988) morphological revision.

We know very little about the dispersal capabilities of this group, let alone the dispersal capabilities of any hypothetical ancestor. Merrick (1983) and Morgan (1997) have noted that the dispersal capabilities are probably very limited within this group.

A few studies have tried to estimate the dispersal capabilities of species within this group. Geddes et al. (1993) used allozymes to infer gene flow within *E. armatus*. They found that this wide-ranging and, in some respects, unusual species (Morgan 1997), had a high level of genetic continuity across its range. Similarly, allozymes were used by Ponniah (1992) to infer the dispersal capabilities of *E. hystricosus*. However, he did not detect enough allozyme variation to be able to make any firm inferences.

We are currently investigating the dispersal capabilities of four Queensland species of *Euastacus* (*E. robertsi*, *E. fleckeri*, *E. hystricosus* and *E. sulcatus*). Our study is focussing on the mode of dispersal (instream vs overland) and the types of habitats that are barriers to dispersal. We are using allozymes, mtDNA and the genetic population structure of the ectocommensal temnocephalans that inhabit these crayfish. Unpublished data for *E. robertsi*, which has three disjunct subpopulations, indicates that this species has very limited dispersal capabilities between populations on different mountains, and even between streams on the same mountain which are not connected by continuous rainforests.

We are also trying to construct a more robust mtDNA phylogeny by adding CO1 mtDNA sequence data. We will include the remaining species found in Queensland, incorporate sequence data from two individuals from each species, and incorporate a few more southern species so that the Queensland species may be put into context. With the development of a robust molecular phylogeny, the development of a reasonable molecular clock, and a better understanding of the dispersal capabilities of select members within the group, more light may be shed on the evolution of this group.

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APPENDIX A

Aligned sequences of the 16s mtDNA region for some members of the genus *Eustacus* and for *Cherax quadricarinatus*.

```
                          1 111111112  2222222223 3333333334 4444444445
                 1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)   GCCCATTG-G GTTACTAAAA GGCCGCGGTA TAGTGACCGT GCGAAGGTAG
2(E. fleckeri)   .......... .......... .......... .......... ..........
3(E. balanensis) .......... ......T... .......... .......... ..........
4(E. eungella)   ....G..... .......... .......... .......... ..........
5(E. hystricosus) ......... .......... .......... .......... ..........
6(E. setosus)    ....G..... .......... .......... .......... ..........
7(E. sulcatus)   .......... .......... .......... .......... ..........
8(E. suttoni)    .......... .......... .......... .......... ..........
9(E. spinifer)   .......... .......... .......... .......... ..........
10(Cheraxquad)   .......... .G-...G... .......... .TT....... ..........

                                                                      1
                 5555555556 6666666667 7777777778 8888888889 9999999990
                 1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)   CATAATCATT TGTCTTTTAA GGCCGCGGTA TAGTGACCGT TTGGACGAAA
2(E. fleckeri)   .......... .......... .......... .......... ..........
3(E. balanensis) .......... A......... ...A...... .......... ..........
4(E. eungella)   .......... A......... ...A...... .......... ..........
5(E. hystricosus) ......... A......... ...A...... .......... ..........
6(E. setosus)    .......... A......... ...A.....C .......... ..........
7(E. sulcatus)   .......... A......... ...A...... .......... .........G.
8(E. suttoni)    .......... A......... ...A...... .......... .........G.
9(E. spinifer)   .......... A......... ...A...... .......... .........G.
10(Cheraxquad)   .......... A......... ...A.....G .A........ .........G.

                 1111111111 1111111111 1111111111 1111111111 1111111111
                 0000000001 1111111112 2222222223 3333333334 4444444444
                 1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)   AGCGATCTGT CTCTA-TGGG AGTAATTGAA TTTAACTTTT GAGTGAAAAG
2(E. fleckeri)   ..T....... .......... .A........ .......... ..........
3(E. balanensis) ..T....... ....G..T.- .A........ .......... ......G...
4(E. eungella)   ..TA...... .......T.A .A........ .......... ......G...
5(E. hystricosus) ..TA...... .......T.. .AA....... .......... ..........
6(E. setosus)    ..T....... ....G..T.A .A........ .......... ......G...
7(E. sulcatus)   ..TA...... .......T.A .AA....... .......... ......G...
8(E. suttoni)    ..TA...... .......T.A .AA....... .......... ......G...
9(E. spinifer)   ..TA...... ....G..T.A .AA....... .......... A.....G...
10(Cheraxquad)   ..GA.G.... .......CTC GAAG...... .......... A.........
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                    1111111111 1111111111 1111111111 1111111111 1111111112
                    5555555556 6666666667 7777777778 8888888889 9999999990
                    1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)      GCTTAAATGG TCTAGGGGGA CGATAAGACC CTATAAAGTT TAACATTATA
2(E. fleckeri)      ........A. .......... .......... .......... ..........
3(E. balanensis)    .......... .......... .......... .......... ..........
4(E. eungella)      .........A .....A.... .......... .......... ..........
5(E. hystricosus)   .........A .....A.... .......... .......... .G....A..G
6(E. setosus)       .........A .....A.... .......... .......... ..........
7(E. sulcatus)      .......... .....A.... .......... .......... .........G
8(E. suttoni)       .........A .....A.... .......... .......... ..........
9(E. spinifer)      .........A .....A.... .......... .......... ...T......
10(Cheraxquad)      .........A GT...A.... .......... .......... ..TACA.G..

                    2222222222 2222222222 2222222222 2222222222 2222222222
                    0000000001 1111111112 2222222223 3333333334 4444444445
                    1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)      GTAGTGAAAG ATTGATT--A AGT---TGTA GAATTTTTT- ATTATGGGTA
2(E. fleckeri)      .......G.. ...A...... .......A.. .GG....... ..........
3(E. balanensis)    A..A.A...A G..A...GG. .-........ AG....A... G....AAA..
4(E. eungella)      A..ACA.T.A G..T...GGG .-......A. AG....A... ...G..AA..
5(E. hystricosus)   ...A.A...A G......... G.......G. .G.C..A... ...GCAAA..
6(E. setosus)       AC.ACA.G.A G..A...GGG .-......C. A.GA...A.T ...G..AA..
7(E. sulcatus)      ...A.A...A G......... G.......GG ..GC..AC.. .....AAA..
8(E. suttoni)       A..A.A...A G......... G......AG. AG.AC..A.T ...G.AA...
9(E. spinifer)      AC.A.AG..A ...A.....- G.A....TAT A.GA...A.T ...G.A...G
10(Cheraxquad)      ..T.GTT... .G.....TA. G.....GT.. A.G-...A.T .CC.GCA.-G

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                    5555555556 6666666667 7777777778 8888888889 9999999990
                    1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)      GTGTTTTGTT GGGGCGACAA CGATAAGACC CTATAAAGTT TTCTTTTTTG
2(E. fleckeri)      .......... .......... .......... .......... ..........
3(E. balanensis)    .......... .......... .......... .......... ..........
4(E. eungella)      .......... .......... .........C .......... ..........
5(E. hystricosus)   A......... .......... .......... .......... ..........
6(E. setosus)       .......... .......... .......... .......... ..........
7(E. sulcatus)      A......... .......... ...C...... .......... ..........
8(E. suttoni)       .......... ........C. .G.C?..... ........?. ..........
9(E. spinifer)      ..A....... .......... .......... .......... ..........
10(Cheraxquad)      ......A... ........T. .G........ T..A...... ..T.C.G..T

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                    0000000001 1111111112 2222222223 3333333334 4444444445
                    1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)      TA--ACAAAT ATGTTTGGGT TAATGATC-T TTTTAAGAGT ATTAGAGTAA
2(E. fleckeri)      ....T..... .......A.. .......... .......... ..........
3(E. balanensis)    ....T..... ..A....AA. .......... .......... ..........
4(E. eungella)      ....T..... ..A....... .......... .......... ..........
5(E. hystricosus)   ....T....A ..A....... .......... .......... ..........
6(E. setosus)       ....T....G ..A....A.. .......... .......... ..........
7(E. sulcatus)      ....T....A ..A....A.. .......... .......... ..........
8(E. suttoni)       ....T.C..G .?A....A.. .G.....?.. .......?.. .....?....
9(E. spinifer)      ....T....A ..A....A.. .?........ .......... ..........
10(Cheraxquad)      A.ATCAG.GA TAT..CTTCG ..TGATC.T. ....-..GA. TAG.......
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                    3333333333 3333333333 3333333333 3333333333 3333333334
                    5555555556 6666666667 7777777778 8888888889 9999999990
                    1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)      ATTACTTTAG GGATAACAGC GTAATTTTTT TT-GAGAGTT CTTATCGACA
2(E. fleckeri)      .......... .......... .......... .......... ..........
3(E. balanensis)    .......... .......... .......... .......... ..........
4(E. eungella)      .......... .......... .......... .......... ..........
5(E. hystricosus)   .......... .......... .......... .......... .C........
6(E. setosus)       .......... .......... .......... .......... ..........
7(E. sulcatus)      .......... .......... .......... .......... .C........
8(E. suttoni)       .....?.... ........C. .......... ..T....... .C........
9(E. spinifer)      .......... .......... .......... .......... .?........
10(Cheraxquad)      .......... .......... .......... ...A...... ..........

                    4444444444 4444444444 4444444444 4444444444 4444444444
                    0000000001 1111111112 2222222223 3333333334 4444444445
                    1234567890 1234567890 1234567890 1234567890 1234567890
1(E. robertsi)      AGAAAGTTTG CGACCTCGAT GTTGAATTAA AGATTCTTTA TA-GTGTAGG
2(E. fleckeri)      .......... .......... .......... .......... .........A
3(E. balanensis)    .A.G...... .......... .......... .......... ...A..C..A
4(E. eungella)      .A.G...... .......... .......... ..G....... ...A..C..A
5(E. hystricosus)   .A.G...... .......... .......... .......... .G.......A
6(E. setosus)       .A.G...... .......... .......... .......... ...A.....A
7(E. sulcatus)      .A.G...... .......... .......... .......... ...A..C...
8(E. suttoni)       .A.G...... .......... .......... .......... ...A.....A
9(E. spinifer)      .A.G...... .......... .......... .......... ......C..A
10(Cheraxquad)      .A.G...... .......... .......... .A...TC.CT G?G.....T

                    4444444444 4
                    5555555556 6
                    1234567890 1
1(E. robertsi)      TGCTATAGAA G
2(E. fleckeri)      C......... .
3(E. balanensis)    A.T....T.. .
4(E. eungella)      A.T....T.. .
5(E. hystricosus)   A.T....T.. .
6(E. setosus)       A.T....T.. .
7(E. sulcatus)      A.T....T.. .
8(E. suttoni)       A.T....T.. .
9(E. spinifer)      A.T....T.. .
10(Cheraxquad)      A.T..C.... .
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